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SCIENCE

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son-on-Hudson, N. Y.

RECENT ADVANCES IN PHYSIOLOGICAL CHEMISTRY.¹

THE enormously rapid development of physiological chemistry in the last ten years may be well illustrated by a consideration of the new journals which have

¹ Read at Buffalo meeting of the American Chemical Society, June 22, 1905.

been found necessary to publish the increasing literature, and also by the influence which this department of science is exerting on other sciences, particularly on medicine. For twenty years the *Zeitschrift für physiologische Chemie* and *Maly's Jahresbericht* were sufficient to represent the specialty, which had been considered a somewhat narrow one, but suddenly, and almost simultaneously, three other publications were called into existence to keep pace with the newly aroused interest. These are the *Biochemisches Centralblatt*, the *Beiträge zur chemischen Physiologie und Pathologie* and the volume on Biochemistry of the *Ergebnisse der Physiologie*, all since 1902.

Several causes have worked to bring about this situation and the most important will be touched on in what follows, but at the outset two things are apparent; on the one hand, there is the stimulating influence of pure organic chemistry, and on the other the requirements of physiology and pathology for a more rational chemical foundation. This last factor is an extremely potent one; some of the most interesting problems of physiological chemistry have been suggested by questions growing out of the discussion of the modern doctrines of immunity and the causation of disease.

It follows, therefore, that many of the advances in physiological chemistry are in lines which are comparatively new, but there are some noteworthy exceptions and of one of these I wish to speak first. This is concerned with the question of protein

in nutrition which has been a much debated problem for fifty years. Indeed, interest in this goes back to the days of the epoch-making publications of Liebig on the relation of organic chemistry to physiology and pathology, issued in the early forties. In these he developed his idea of the functions of various foods in the nutrition of man and laid particular stress on the importance of protein as the source of muscular energy. According to this early Liebig view our foods may be divided into plastic or tissue-forming, on the one hand, and heat-producing, on the other. The production of heat appeared as an end in itself and the fats and carbohydrates served for this purpose. The protein substances are built up into tissues and in the oxidation of the latter, it was held, we have the sole source of muscular energy. The name of Liebig was all-potent in science in those days and his nutrition theory held sway for twenty years or more without question. It will not be necessary to recount the steps in the opposition which finally developed, but it may be well to recall the famous experiment of Fick and Wislicenus in which, in an ascent of the Faulhorn, in 1866, they calculated the work done and the protein oxidized, as measured by the urea excretion. The protein combustion was found to be far too little to account for the expenditure of work in the climb, which result confirmed the theoretical objections urged, especially by J. R. Mayer, of Heilbronn. Other important investigations followed in the same direction, and almost without exception they have gone to show that while the protein oxidation *may* furnish a part of the muscular energy of the body, or even all of it under certain extreme circumstances, the fats and carbohydrates are the usual sources of such energy in man, and that heat production is only incidental, not an end, but an unavoidable accompaniment. A few recent experi-

ments which have seemed to support the Liebig contention have been made largely with carnivorous animals and have no real bearing on the problem as far as man is concerned.

As a necessary consequence of the Liebig theory it was held that our protein consumption must be high, and hence the large amounts of nitrogenous substances insisted upon in the older dietaries. But after a time physiologists naturally began to inquire into the real uses of protein, if it is not called for in the work of the muscles; if, as appeared evident, it is used mainly in the repair of waste tissues, why metabolize so much, since in this metabolism an enormous amount of extra work is thrown on the oxidizing and excreting organs of the body. It certainly can not be assumed that the disposal of the katabolic products of proteins can be accomplished without using up a considerable amount of energy, and without a great strain on the liver and kidneys. What, then, is the amount of protein actually needed for the normal body? Numerous answers have been given to this question and in late years several investigations have apparently brought the daily protein down to 25 to 40 grams, or even lower. But it has been urged against all the experiments leading to such results that they were of too short duration to actually prove anything of value. For example, Siven carried out a 32-day test in which the protein metabolized daily was about 38 grams; during a part of this time the body was kept in nitrogen equilibrium by about 25 grams daily. Hirschfeld somewhat earlier had made numerous observations in which the protein consumption through about two weeks was 35 to 45 grams, but fats and carbohydrates brought the diet up to an equivalent of 3,750 to 3,900 calories.

The importance of the subject is worthy of the fullest investigation, and such a

study has finally been carried out by Chittenden through experiments, first on himself, and then on groups of men engaged in various occupations. In the first of these remarkable experiments, which have recently been described in book form under the title 'Physiological Economy in Nutrition' the distinguished Yale scientist determined in his own case how far he could safely reduce the protein of his diet and still retain the body in nitrogen equilibrium. To do this close watch was held on the food and excreta through a year, November, 1902, to October, 1903, and under varying external conditions of work and temperature. As a result of these systematic tests Chittenden found that he could live very comfortably, and in perfect health, on a diet containing 35 to 40 grams of protein daily, with fats and carbohydrates sufficient to yield 1,500 to 1,600 calories. These valuable personal experiments were regarded as preliminary only. Later, systematic observations were made with three groups of men, the work being carried through periods of five to nine months for each group.

The first group comprised colleagues of the author of the experiments, Yale professors and instructors. The average protein metabolism here was about 46 grams. The second group was composed of soldiers from the hospital corps of the United States army who were detailed for the purpose of the study. Of the twenty who began, thirteen followed the tests through the whole period of over six months. Those who deserted, or were dropped, had much to say through the newspapers about starvation diet, but this was a curious misnomer, since, as the records show, the men who remained were kept in perfect nitrogen equilibrium and found themselves in far better physical condition at the end of the experiments than at the beginning. Through all this time they had plenty of

work to perform, with constant and rather severe requirements on the muscular system. The average protein consumption daily was not far from 55 grams.

Finally, eight Yale athletes showed themselves willing to work through the training and competing season on the restricted protein diet. The results here were equally remarkable, in fact probably the most remarkable, as the work done by these men was of a character to call for very high protein diet according to all of our old standards. The experiments were carried out through a period of five months, February to June, 1904, and through the last two months a very close record was kept of diet, excretion, weight and various other factors concerning the men. Through this sixty-day period, when the muscular exertion was, perhaps, the most taxing, protein equilibrium was maintained on an average of 8.81 grams of nitrogen metabolized for each man daily, corresponding to about 55 grams of protein. All these men took high rank in athletic work, several of them being prize winners. The reproductions of photographs, published in the book, show them to be men of excellent physique, and even of remarkable muscular development in some cases. While the protein diet of these men was low the fat and carbohydrates were generous but not excessive, the calorific value of the whole being seldom over 3,000 calories.

For all these men under examination in these three sets of tests, professional men, soldiers, athletes, complete statistics for each day are published, from which the reader may derive the fullest possible information. Painsstaking accuracy is evident in every page, and from the standpoint of logical requirement in experimental proof the tables meet any reasonable objection.

This Chittenden investigation then must

be regarded as of fundamental importance, as it demonstrates beyond cavil just what is possible in protein restriction under ordinary conditions. The periods of investigation chosen were long enough to answer objections to the results of some of the earlier tests, and the values obtained for the soldiers and athletes of about 55 grams of protein metabolized daily will have to be taken as practical standards. It doubtless remains true that for men at severe work at low temperatures a large number of calories are required in the food. An instructive example of such dietaries is given in the recent publication by C. D. Woods on the diet of Maine lumbermen, where it is shown that the heat value of the food consumed daily by men in the lumber camps may amount to 6,000 or 8,000 calories. It would be interesting to experiment in such cases on the replacement of a good share of the protein by fat and carbohydrates.

A study of the Chittenden series of experiments on men shows very clearly that as far as the human organism, at any rate, is concerned the old Liebig notion of the source of muscular energy is without foundation. As suggested above, experiments with carnivorous animals do not apply to man; it would be as justifiable to discuss the food value of pentoses for man from experiments on the feeding of straw to cattle. It is true that for short periods, or under special conditions, proteins may serve man as the main or only source of muscular energy, but evidently this is not usually or normally the case.

When the far-reaching importance of the whole question is realized, and when it is further remembered that considerable internal work must be done to remove, especially, the products of protein metabolism, I believe it will be granted that I am right in placing this work of Chittenden

among the most important recent achievements in physiological chemistry.

The next topic of which I wish to speak very briefly deals with a problem even older than that of the Liebig theory of the source of muscular energy. Some years before the organic chemistry of Liebig was published Mulder had introduced the term protein, and had even announced the essential composition of what he considered the protein nucleus. His positive statements led to extended investigations on the part of others, and the work of many chemists soon disclosed the fact that no one simple nucleus may be assumed to exist in these molecules and that they must be enormously complex. Ever since the early forties the problem has been an extremely interesting one, but it is only recently that it has been seriously attacked from the second side possible in such investigations. Up to a period within five years the work done on the protein question has been largely in the way of analysis or disintegration, but now we have the beginning of attempts at synthesis or reconstruction of large groups. Glycine and leucine had been known since about 1820 as decomposition products of glue and other bodies by action of acid. Nearly thirty years later tyrosine was added as obtained in about the same way, and soon a few other individual substances were listed among the products which could be secured in various decompositions of proteins. In the seventies systematic methods of hydrolysis by alkalis and acids were worked out, especially by Schützenberger and Hlasiwetz and Habermann. Numerous products were recognized, but at first these attracted no great attention, as there remained always the possibility that the amino acids and other compounds found might be results of secondary reactions. We can not infer much regarding the structure of soft coal from the presence of methane in the gas, or of benzene, tolu-

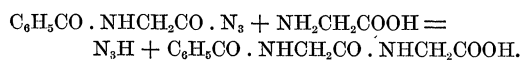
ene and naphthalene in the tar, and an analogous proposition may be true for the proteins. Later, however, this important generalization was reached; it matters little how the decomposition is effected, the products of protein destruction are essentially the same as long as brought about in the presence of water, and all seem to be in the nature of hydrolytic cleavages. The action of boiling acid or alkali, steam under pressure, pepsin and hydrochloric acid, trypsin and weak alkalies, all lead to nearly the same resultant products, and among these certain α -amino acids are always the most abundant. The conclusion follows, therefore, almost of necessity, that these are the true nucleus groups and the question naturally suggests itself, is it possible to put these things together and build up anything like a true protein. An answer to the question has been slow in coming, but a beginning has been made, and especially through the experiments of Curtius and Fischer. The condensation method followed by the former is a general one, through which a large number of amino groups have already been combined. It depends first on the production of hydrazides, then azides, which are very reactive, and take on additional amino groups with loss of hydronitric acid. For example, the ethyl ester of hippuric acid condenses with hydrazine hydrate to form the hydrazide:



this with nitrous acid gives the azide



By treatment with glycine under certain conditions this reaction follows:



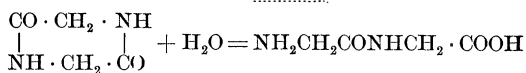
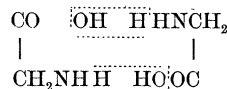
In other words, we have started with benzoylglycine and have obtained benzoylglycylglycine. This in turn may be used as a new starting point. A silver salt is made,

this turned into ester with ethyl bromide, and then a new hydrazide and azide to be combined with glycine, as before. These steps will lead to benzoyl diglycylglycine, and by using alanine, leucine or other amino acid it will be seen that by repeating the processes extremely complex groups may be finally built up. Curtius has carried the reaction to the formation of benzoyl hexaglycylglycine,



Hippuryl and three alanine groups have also been condensed to form benzoyl glycylalanylalanine.

Fischer has worked from a different standpoint. The study of various hydrolytic products from proteins, already referred to, and the isolation of certain other groups by Fischer himself, led to the belief that the complex molecules in the proteins must be built up by the union of amino acid groups. Various attempts had been made to condense some of the simple amino acids in anhydride form, but without much success, until the first experiments were made by Fischer in 1901. The starting point of the series of condensations was found in the product obtained from the glycine anhydride described in 1888 by Curtius and Goebel. This may be looked upon as formed by the union of two molecules of glycine with loss of two molecules of water, and when digested with strong hydrochloric acid suffers a peculiar decomposition and yields a body to which Fischer has given the name glycylglycine.



Glycylglycine may be considered as the first anhydride of glycine and is the characteristic group in the hippuryl glycine

from which Curtius has made the derivatives referred to above. In the same connection Fischer explained that a similar reaction follows with the anhydrides of alanine and leucine, from which alanylalanine and leucylleucine must result. To carry the process farther and obtain more complex groups proved practically difficult. Another method was finally developed which may be illustrated by some of the simplest cases described by Fischer. On bringing together a halogen acid chloride, for example chloracetyl chloride, with an ester of glycylglycine, chloracetylglycylglycine ester results,



which on saponification yields the acid. The latter in turn when treated with strong ammonia gives up chlorine for the amino group and diglycylglycine results. With α -bromopropionyl bromide employed as the halogen compound, alanylglycylglycine is obtained, and with α -bromisocapro-nyl chloride leucylglycylglycine is secured in the same manner.

It will be seen that while the processes of Fischer and Curtius follow different lines they lead usually to the same ends. A considerable number of the Curtius investigations have been published in Vol. 70, N. F., 1904, of the *Jour. prakt. Chem.* under the title of 'Condensations of Amido Acids,' while the Fischer work has come out in recent volumes of the *Berichte* under the general title of 'Syntheses of the Polypeptides.' This work seems to be more directly concerned with the building up of bodies of physiological interest; the Curtius work is somewhat more general. Of many of the Fischer compounds it has been shown that ready hydrolysis with active pancreatic juice follows. The biuret reaction is also given in many cases, but apparently not always. Groups containing tyrosine, cystin, leucine, alanine, etc., have

been split by the ferments, and these, it will be remembered, are among the most important of the fractions secured by the hydrolysis of the true proteins. The method of producing these polypeptides seems to be without limit and doubtless much more complex aggregations will be secured. Molecular weights of over 500 have already been reached.

Closely related to the question of the synthesis of the polypeptides is that of the composition of the simplest proteins. The work of Kossel and others in this direction has furnished most interesting results. For several of the protamines and histones the content of hexone bases has been found with a fair degree of accuracy, and of many of the more complex proteins the amounts of both mono- and diamino acids present have been found. The numbers secured must be looked upon, however, as minimum values because of the practical difficulties in the way of quantitative separation and identification.

Several improved processes have been developed for the separation of amino acids from digestion or other mixtures. A method first suggested by Curtius for the production of ethyl esters of the amino acids, and which has been referred to above, has been perfected by Fischer. From mixtures the esters are distilled off under greatly reduced pressure. From the distillate some are separated by solvents, while others, after conversion to acids, are separated by fractional crystallization. It has been found that β -naphthalene sulphochloride combines with many of the amino acids to form compounds of very slight solubility and Fischer and Bergell have developed a method of separation based on this fact. Both general methods have been applied also in the detection and estimation of amino acids in urine which is likely to become a matter of considerable clinical in-

terest, as these acids must represent degenerative or otherwise abnormal products having their origin in the liver and other organs.

Unquestionably one of the most important fields of effort in physiological chemistry at the present time is the study of the soluble ferments, and we have here for consideration not only certain newly discovered enzymes, but, perhaps, of more importance, a great advance in our knowledge of those already known. It was not many years ago that we considered the question of the gastric enzymes as practically settled. The presence of both pepsin and rennin no one could have doubted, but the work of Pawlow and his school in the last five years has thrown entirely new light on the subject and it would appear that one and the same ferment, working under different conditions, is responsible for both classes of observed phenomena. Pawlow has compared the digesting and milk curdling power of ferment secretions from the true pepsin and pyloric glands of the stomach, from the pancreas and from Brunner's glands, and has found them perfectly parallel under proper conditions of experiment; any cause which operates to destroy one power, destroys also the other according to Pawlow. But in any given extract or preparation the conditions must be properly chosen to show both effects. A commercial rennet, for example, may exhibit the milk curdling action very strongly, yet appear to be wholly inert toward fibrin. Pawlow holds that in all such cases simple dilution with very weak hydrochloric acid is all that is called for to show the peptic power. A valuable résumé of this work is given in a recent number of the *Zeitschrift für physiologische Chem.* (Vol. 42, p. 415, 1904).

It is proper to say that the physiologists of the Hammarsten school do not admit the

claims of Pawlow, although the doctrines of the latter have been put in very strong light.

To Pawlow we owe, also, the discovery of a new group of ferments which he calls kinases, or activators. The most thoroughly studied of these is the enterokinase which converts the inactive pancreatic juice into an active ferment secretion. As to the value of the other kinases much less is known.

About four years ago Cohnheim described, under the name of erepsin, a peculiar ferment in the intestinal walls which has the power of splitting peptones and proteoses, but not albumins. This discovery grew out of an investigation to determine the fate of the peptone bodies of proteolysis, it being long known that no appreciable amount of these substances appears in the circulation after digestion. A theory grew up to the effect that in the intestinal walls, just before absorption, they were converted back into true albumins. According to the views now advanced by Cohnheim and others this can not be the case to any large extent. The peptones break down with liberation of the carbon and hydrogen excess, which serves as a source of energy, while the nitrogen fractions go over into the form of amino acids, to be further broken down by the liver. This coincides with the view that very little protein is actually needed by the body. On the other hand, it is held by several physiologists that the erepsin katabolism does not go so far, but merely to the production of amino compounds which are ready for a synthesis not yet understood.

If time permitted I should like to go into the question of enzymes in other directions, especially with reference to the work in the liver and the action of the so-called autolytic ferments, the behavior and general importance of which are still very ob-

seure. A large and interesting literature has grown up around the discussion. A word must be said, however, about the important discussion started two years ago by Cohnheim when he announced the relation of two distinct ferments to the oxidation of carbohydrates in the body. The bearing of this on the question of diabetes was immediately recognized and numerous investigations were launched to throw more light on the subject. According to Cohnheim the pancreas furnishes one of these enzymes and the muscle substance the other. One may serve as a kinase or activator for the other and the effect of the two is to facilitate oxidation in the muscles. The subject is immensely important, but the latest studies do not seem to fully confirm all the Cohnheim statements.

In connection with the subject of enzymes reference must be made to the considerable number of papers which have appeared in the last few years on the question of the relation of the ferments to the simple inorganic catalytic agents. Beginning with the work of Tammann published in the *Zeitschrift für physikalische Chemie* many attempts have been made to express the velocity of enzyme reactions by equations analogous to those suggested by Wilhelmly for the inversion of sugar. The extended investigations of Tammann led in general to formulas which were more complicated than those corresponding to the simple logarithmic curve. Some of the more recent work, especially that of Henri, has led to more definite results. This whole discussion has been well reviewed by Bredig in volume 1 of the *Ergebnisse der Physiologie*.

One of the most interesting developments in recent physiological chemistry is in the discussion of theories of immunity and the relations of toxins and antitoxins. As first presented by Buchner, Bordet, Ehrlich,

Pfeiffer and others, these doctrines appeared from the chemical standpoint wholly visionary and intangible, but in the last few years a great change has followed in the attitude of chemists and now some of the phrases of the immunity theory of Ehrlich have become part of the language of organic chemistry.

It was early recognized that toxins and antitoxins act on each other in a manner suggesting combinations in definite chemical proportions, and attempts were soon made to work out the laws of the union. The earlier Ehrlich experiments seemed to point to simple combinations like those between an acid and a base, the union following to complete saturation. It was recognized later, however, in many cases, that the reaction is not complete and that the saturation curve is far from being a straight line. These observations led to various speculations. Ehrlich assumed that in the ordinary toxin mixtures we have certain modified forms known now as toxoids and toxons, which, while non-toxic, have saturating power resembling that of the toxins. Hence the amount of antitoxin added to a toxin solution to destroy its toxicity would have to be sufficient to combine, not only with the real toxin, but with any toxoid or toxon present also; just as in neutralizing free sulphuric acid by sodium carbonate the amount of the latter necessary would have to be increased if some salt decomposable by sodium carbonate, such as alum, is likewise present. In the one case as in the other the simplicity of the reaction would be obscured by complexity of the mixture.

Arrhenius and Madsen, and others following them, have been led also to study these extremely important phenomena and have given a different interpretation. According to the notions of the physical chemists these reactions are more or less per-

fectly reversible, which certain experiments seemed to prove, and resemble somewhat the union of an alcohol and an acid which combine to reach a condition of equilibrium. They assume for the toxin-antitoxin reaction the perfect applicability of the Guldberg-Waage mass action formulas, and for a number of relations have calculated the value of the constant k . It is interesting to note that a number of the leading physical chemists have taken part in the discussion. About a year ago Michaelis reviewed the subject in a long article in the *Biochemisches Centralblatt* and this has recently appeared in expanded book form under the title, 'Die Bindungsgesetze von Toxin und Antitoxin.' Michaelis does not accept the Arrhenius work as satisfactory or convincing, and points out several conditions necessary for the applicability of the mass action laws which do not obtain in the cases in question; for example, the mixtures are not homogeneous and the degree of reversibility is extremely limited, if it really exists.

On the other hand, the doctrine of the toxoids and toxons appears to explain the apparent discrepancies and in certain mixtures secured in the experiments of Keyes and Sachs, known to be free from these bodies, the toxin and antitoxin combination followed in proportions represented by an almost perfect straight line.

It remains to add that this whole discussion can not fail to have an important influence on the attitude of medical men to the rapidly developing physiological chemistry. The Arrhenius theory seemed to simplify the question somewhat and make it one of analogy with other well-known phenomena. The facts more recently adduced by the Ehrlich workers do not seem to permit this theoretically preferable solution. The toxoid and toxon hypotheses are necessarily chemical, however, and for the

present may better serve in the advance of investigation.

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SCIENTIFIC BOOKS.

The Evolution of Man. By ERNST HAECKEL. Translated from the fifth German edition by JOSEPH McCABE. 2 vols., 8vo. New York, G. P. Putnam's Sons. 1905.

In the two stately and richly illustrated volumes before us we have a translation of the fifth edition of Haeckel's 'Anthropogenie,' and coming as they do from the pen of one who may now be regarded as a Nestor of zoology and the most vigorous exponent of the historical method of investigation, they present not a little interest. They profess to give in their course of some nine hundred pages an account of the embryological and comparative anatomical evidence bearing on the origin of man, a subject of perennial interest not only to the laity, but also to professional zoologists, since it involves the problem of the origin of the vertebrates.

The work opens with a chapter upon the biogenetic law, or, as it is termed, 'the fundamental law of organic evolution,' and then follow five especially interesting chapters devoted to a history of the development of embryology and phylogeny. To these succeed an extended account of the principal embryological stages of the vertebrates and a discussion of their significance, in which the germ cells, segmentation, gastrulation, the germ layers, metamerism, the fetal membranes and the development of the general form of the body, are all considered from the standpoint of their bearings on the ancestral history. This completed, the author passes on to a consideration of the recent representatives of the ancestral stages and concludes with several chapters devoted to the phylogeny of the various organs of the human body.

It would require much space to consider adequately the entire contents of the volumes, and the purpose of this review will, perhaps, be best served by indicating briefly the line of descent which Haeckel advocates. It is essentially the same as that presented in earlier